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Adjuvant mixture.

㉒ According to the present invention oil-free vaccines are provided which contain polyoxypropylene-polyoxyethylene polyols as well as an acrylic acid polymer as adjuvating constituents. These vaccines were found to show excellent immunizing properties.

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The invention relates to an oil-free adjuvant mixture which contains polyoxypropylene, polyoxyethylene polyols, and to a vaccine which contains such an adjuvant mixture. For protection against transmittable infectious diseases it is usual to vaccinate humans and animals with immunogenic material against which protective antibodies can be formed.

In the last two cases it is necessary for one or more components which stimulate the immune response of the host to be protected to have been added to the antigen as well.

Hunter et al. [R. Hunter, F. Strickland & F. Kézdy (1981) *J. Immunol.* **127** (3), 1244-1250; H. Snippe, M.J. de Reuver, F. Strickland, J.M.N. Willers and R.L. Hunter (1981), *Int. Archs. Allergy appl. Immunol.* **65**, 390-398; R.L. Hunter and B. Beninett (1984) *J. Immunol.* **133**, 3167-3175] described the use of non-ionic block polymers in vaccines.

$$\text{HO}(-\text{CH}_2-\text{CH}_2-\text{O})_{\underline{a}}-(\underset{\text{CH}_3}{\text{CH}}-\text{CH}_2-\text{O})_{\underline{b}}-(\text{CH}_2-\text{CH}_2-\text{O})_{\underline{c}}-\text{H} \quad (\text{I})$$
$$\text{HO}-(\text{CH}-\text{CH}_2-\text{O})_{\text{d}}-(\text{CH}_2-\text{CH}_2-\text{O})_{\text{e}}-(\text{CH}-\text{CH}_2-\text{O})_{\text{f}}-\text{H} \quad (\text{II})$$

$\begin{array}{c} | \\ \text{CH}_3 \end{array}$

$\begin{array}{c} | \\ \text{CH}_3 \end{array}$

In addition to these so-called "triblock" copolymers, the use of so-called Tetric® polyols as block 40 polymer surfactants was described by Hunter & Bennett (1984). These are also termed "octablock" copolymers, and consist of a core of 4 polyoxypropylene or polyoxyethylene chains to which polyoxyethylene or polyoxypropylene chains respectively are bonded at the 4 ends. These are the so-called T-type polyols.

The object of the present invention is therefore to provide an oil-free adjuvant mixture which is more
50 potent than the above-named mixtures which contain only block-polymer polyoxypropylene:polyoxyethylene
polyols.

The adjuvant according to the the invention. is characterized in that, in addition to polyoxypropylene/polyoxyethylene block polymers it also contains at least one acrylic acid polymer.

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The ratio of the hydrophobic to hydrophilic sections of said block polymers affects the physical constitution (liquid, paste or solid) to a considerable degree. The compounds from the Pluronic series which are suitable are, in particular, Pluronics from the liquid series (L, R or T) and the paste series (P), with the preference being for Pluronics from the liquid series. The preference for Pluronics from the liquid series is disposed towards Pluronic L 101, Pluronic L 121, Pluronic 25R 1, Pluronic 31R 1 and Pluronic T 1501.

The concentration of the block polymers in vaccines according to the invention is preferably approximately 0.1-10% on a weight basis, and in particular approximately 2.4-4.8% on a weight basis.

The term acrylic acid polymer means carbomers such as carboxypolymethylene or carboxyvinyl polymer.

Preferably, a polyacrylic acid is used which is crosslinked with polysaccharide, and in particular with polyallyl sucrose. The properties of such polyacrylic acids are determined by the number of carboxyl groups which is present in the final molecule. Suitable acrylic acid polymers are, for example, the substances which are known under the brand names Carbopol, Neocryl and Carbo-set. The preference is disposed towards Carbopols, such as Carbopol 934, Carbopol 907, Carbopol 910, Carbopol 940 and Carbopol 941.

The concentration of acrylic acid polymers in vaccine mixtures according to the invention is preferably 0.01-2% on a weight basis, and more particularly, 0.015-0.5% on a weight basis.

The ratio of the polyoxypropylene/polyoxyethylene block polymers to the acrylic acid polymers is preferably between 160:1 and 5:1, and in particular around approximately 16:1, on a weight basis.

As indicated above, the adjuvant mixtures according to the invention are suitable, in particular, for use in vaccines which contain non-living antigen material. This may be killed pathogens, or immunogenic fractions (subunits) thereof.

The pathogens may be, for example: viruses, bacteria, or parasites. These may be killed with chemical or physical agents. Here "killed" means inactivation, for example by a change in the genetic material and/or other vital constituents such that the pathogen is no longer capable of multiplying. Suitable chemical agents for killing pathogens are, for example, formaldehyde, glutaraldehyde, β -propiolactone, ethyleneimine and derivatives, or some other compounds which are capable of reacting in a bifunctional or multifunctional manner with reactive groups belonging to the pathogen. Physical agents for killing pathogens are, for example, UV radiation, gamma radiation, "heat shock" and X-ray radiation.

Antigen fractions of the above-named pathogens can be produced therefrom by means of chemical or physical decomposition methods, followed, if desired, by separation of a fraction by means of chromatography, centrifugation and similar techniques. In general low-molecular components are then obtained which, although very pure if desired, will also often have low immunogenic action. If desired, the low-molecular fractions of pathogens may be bonded to a carrier (for example, keyhole limpet haemocyanin or liposomes or other micellar complexes) to increase the immunogenicity. The abovementioned term "immunogenic fractions of pathogens" also means synthetic antigens or haptens, used to mimic natural antigens of the present pathogen. Such synthetic antigens or haptens can be prepared in a known manner by means of organic synthetic methods, or in the case of, for example, polypeptides, by means of recombinant DNA methods.

The concentration of antigen in a vaccine according to the invention is in general 1-95% on a weight basis.

In addition to such a vaccine which contains immunogenic material of only one pathogen (so-called monovalent vaccines), vaccines which contain immunogenic material of several pathogens (so-called combined vaccines) also belong to the invention as well. Such combined vaccines contain, for example, material from various viruses or from various strains of the same virus, or from virus/bacteria combinations or various bacteria.

Example 1

Groups of 10 mice were vaccinated intramuscularly with 0.1 ml of adjuvant mixture containing inactivated Pseudo-rabies Virus (PRV) (10^6 TCID₅₀/ml). Blood samples were taken at 8 and 16 weeks after vaccination, after which antibody titres in the serum were determined by means of ELISA; the results are summarized in table 1.

Table 1

Group	Adjuvant	Antibody response after	
		8 weeks	16 weeks
1	none	10.9±0.9 ^a	9.9±1.1
2	Pluronic L 121 ^b	11.4±2.1	11.1±1.7
3	Carbopol 934 ^c	14.0±1.4	11.7±2.1
4	Pluronic L 121 ^b + Carbopol 934 ^c	15.1±0.8	14.0±1.3

^a) mean ELISA titre (²log) with standard deviation

^b) 4,8% w/v

^c) 0.15% w/v

Example 2

Groups of 10 mice were vaccinated intramuscularly with 0.1 ml of adjuvant mixture containing inactivated PRV (10⁶ TCID₅₀/ml). Blood samples were taken at 6 and 12 weeks after vaccination, after which antibody titres in the serum were determined by means of ELISA; the results are summarized in table 2.

Table 2

Group	Adjuvant	Antibody response after	
		6 weeks	12 weeks
1	none	10.1±1.0 ^a	9.2±1.3
2	Pluronic L 121 ^b	11.5±1.4	11.0±1.5
3	Carbopol 940 ^c	11.7±1.5	11.4±1.1
4	Pluronic L 121 ^b + Carbopol 940 ^c	13.8±1.6	13.2±1.0

^a) mean ELISA titre (²log) with standard deviation

^b) 2,4% w/v

^c) 0.015% w/v

Example 3

Groups of 10 chickens were vaccinated intramuscularly with 0.5 ml of adjuvant mixture containing inactivated paramyxovirus (P₁G strain) (10⁶ EID₅₀/ml). Blood samples were taken at 4 weeks after vaccination. Antibody titres in the serum were determined by means of the haemagglutination inhibition test (HAR); the results are summarized in table 3.

Table 3

Group	Adjuvant	Antibody response (HAR) 4 weeks after vaccination
1	none	0.3 ± 1.0 ^a
2	Carbopol 941 ^b	2.8 ± 2.2
3	Carbopol 941 ^b + Pluronic L 101 ^c	5.6 ± 1.0
4	Pluronic L 101 ^c	0 ± 0
5	Carbopol 940 ^b	2.4 ± 2.0
6	Carbopol 940 ^b + Pluronic L 101 ^c	5.5 ± 1.3

^a) mean HAR titre (²log) with standard deviation

^b) 0.075% w/v

^c) 4.8% w/v

Example 4

Groups of ten mice each were vaccinated intramuscularly with various dilutions of adjuvant containing inactivated PRV (10^{8.0} TCID₅₀/ml). The mice were challenged 4 weeks after vaccination with virulent PRV (Phylaxia strain). The 50% protective doses were determined for the various adjuvants compositions as indicated in table 4.

Table 4

Group	Adjuvant	PD ₅₀ (μl)
1	mineral oil	24.3
2	Pluronic T 1501 ^a	4.0
3	Carbopol 934 ^b	9.2
4	Carbopol 934 ^b + Pluronic T 1501 ^a	5.3

^a 2.4% w/v

^b 0.15% w/v

Example 5

Groups of 7 or 8 pigs were vaccinated with inactivated PRV. The antibody response of the pigs 3 weeks after vaccination was determined by virus neutralization (VN) tests. The 50% protective dose of the various formulations was determined in mice as described in Example 4. The results are summarized in table 5.

Table 5

Group	Adjuvant	PD ₅₀ (μ l) (mice)	VN titre (² log) with standard deviation (pigs)
1	Carbopol 910 ^a	48.5	5.1 \pm 2.4
2	Pluronic T 1501 ^b	55.7	4.0 \pm 2.3
3	Carbopol 934 ^a + Pluronic T 1501 ^b	33.0	5.5 \pm 2.4

^a 0.5% w/v^b 2.4% w/vExample 6

Analogous to the previous examples pigs were vaccinated against Escherichia coli pilus antigens with various vaccine compositions containing 55 μ g K88ab antigen/ml. At 3 weeks post-vaccination the antibody response was measured by ELISA. The results are presented in table 6.

Table 6

Group	Adjuvant	Antibody titre (² log) with standard deviation
1	Pluronic T 1501 ^a	8.0 \pm 1.2
2	Carbopol 934 ^b	8.7 \pm 1.0
3	Pluronic T 1501 ^a + Carbopol 934 ^b	9.7 \pm 1.8

^a 2.4% w/v^b 0.15% w/vClaims

1. Oil-free adjuvant mixture which contains polyoxy-propylene/polyoxyethylene polyols, characterized in that the mixture also contains at least one acrylic acid polymer.

2. Oil-free adjuvant mixture according to claim 1, characterized in that said mixture contains carbomer as acrylic acid polymer.

3. Vaccine, characterized in that it contains an oil-free adjuvant mixture according to claim 1 or 2 and also immunogenic material.

4. Vaccine according to claim 3, characterized in that it contains the said adjuvant component(s) in a quantity of 1-30% referred to the total weight.

5. Vaccine according to claim 3 or 4, characterized in that it contains immunogenic material from an animal pathogen.

6. Vaccine according to claim 5, characterized in that it contains a fowl pathogen as animal pathogen.



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EUROPEAN SEARCH REPORT

Application Number

EP 88 20 0443

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
X	US-A-3 178 350 (LUND) * Whole document *	1-6	A 61 K 39/39
X	US-A-3 869 546 (LUND) * Whole document *	1-6	
X	US-A-3 919 411 (GLASS) * Whole document *	1-6	
X	WO-A-8 500 014 (AMERICAN HOME PRODUCTS CORP.) * Page 12, lines 8-18; page 45, example 11 *	1-6	
A	EP-A-0 135 376 (SYNTEX) * Whole document *	1	
A	EP-A-0 121 752 (BEHRINGWERKE) * Whole document *	1	
D,A	THE JOURNAL OF IMMUNOLOGY, vol. 127, no. 3, September 1981, pages 1244-1250, The American Association of Immunologists, US; R. HUNTER et al.: "The adjuvant activity of nonionic block polymer surfactants. I. The role of hydrophile-lipophile balance"		TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
D,A	THE JOURNAL OF IMMUNOLOGY, vol. 133, no. 6, December 1984, pages 3167-3175, The American Association of Immunologists, US; R.L. HUNTER et al.: "The adjuvant activity of nonionic block polymer surfactants. II. Antibody formation and inflammation related to the structure of triblock and octablock copolymers"		A 61 K
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 26-04-1988	Examiner SKELLY J.M.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date I : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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